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Improved enzyme kinetic model for nitrification in soils amended with ammonium

I. Literature review

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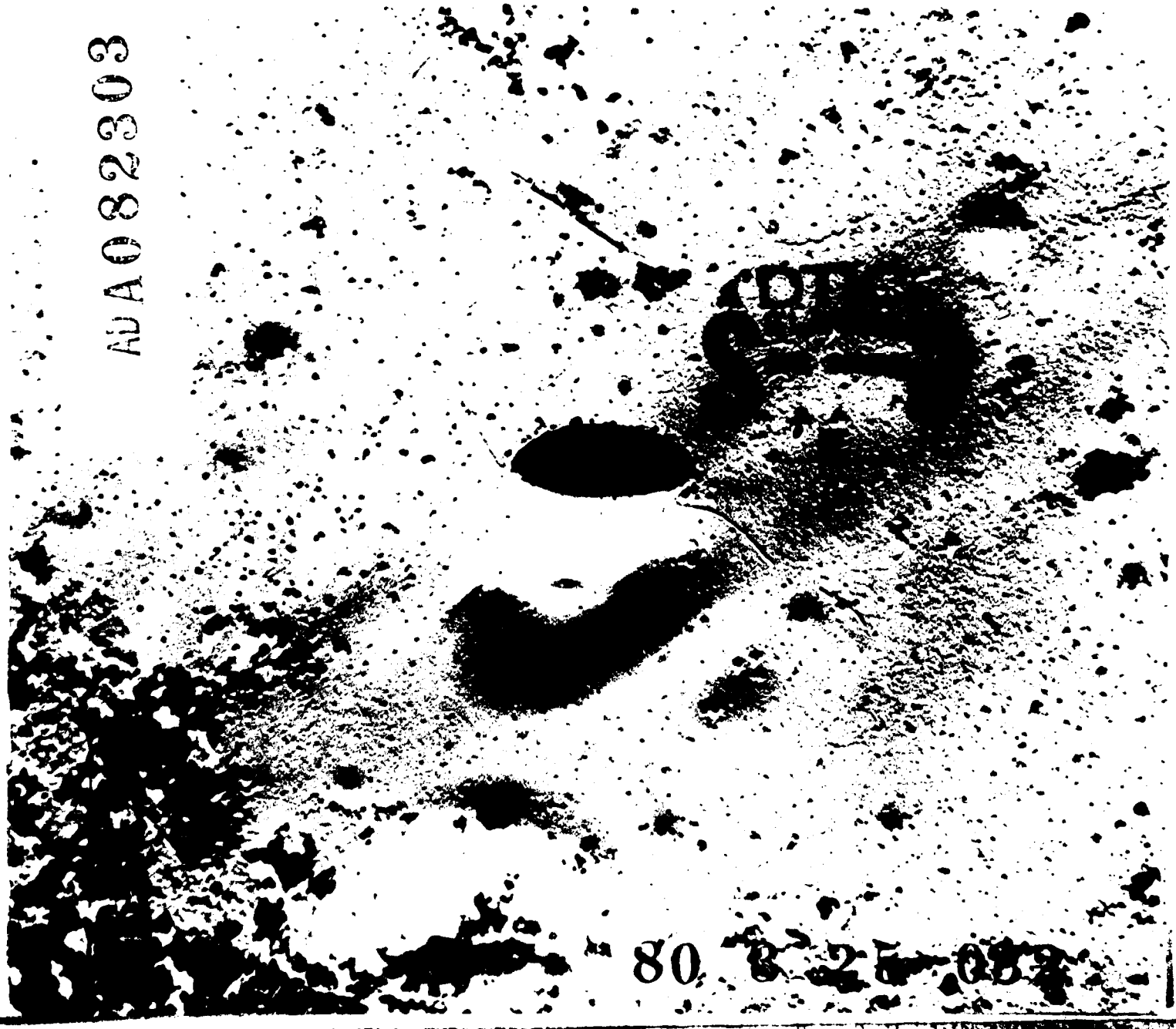

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Improved enzyme kinetic model for nitrification in soils amended with ammonium

I. Literature review

Daniel C. Leggett and Iskandar K. Iskandar

January 1980

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Previous research indicates that nitrification in pure cultures can be represented by Michaelis-Menten kinetics. However, the effects of temperature and especially pH have not been treated systematically in any of the previous reviews of the subject. The work reported here is an attempt to synthesize reported temperature and pH effects on nitrification and nitrifier growth rates. In addition we attempt to extend the principles of microbial kinetics to soils. Our work indicates that pH effects can be interpreted mechanistically as inhibitions by hydrogen and hydroxyl ions, nitrous acid, the ammonia. These are incorporated into the Michaelis-Menten expressions. It is also our observation that ammonium oxidizers in natural habitats are characterized by lower Michaelis constants than pure cultures. This is significant particularly in terms of their growth and activity in acid soils. Alternatively, we speculate that prolifer-

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ation of ammonium oxidizers in acid soils is due to spatial heterogeneity of "pH" at the microsite level.

PREFACE

This report was prepared by Daniel C. Leggett, Research Chemist, and Dr. Iskandar K. Iskandar, Research Soil Chemist, of the Earth Sciences Branch, Research Division, U.S. Army Cold Regions Research and Engineering Laboratory. Funding was provided by U.S. Army Corps of Engineers Civil Works Project CWIS 31314, *Nitrogen Transformations in Land Treatment Systems.*

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IMPROVED ENZYME KINETIC MODEL FOR NITRIFICATION IN SOILS AMENDED WITH AMMONIUM

I. LITERATURE REVIEW

Daniel C. Leggett and Iskandar K. Iskandar

INTRODUCTION

Although nitrifiers and nitrification have been the subject of many investigations, not until recently have attempts been made to simulate this important process in soils. The kinetics have, in different instances, been described as first order (Cameron and Kowalenko 1976, Duffy et al. 1975, Mehran and Tanji 1974, Misra et al. 1974, Starr et al. 1974), zero order (Beek and Frissel 1973, Sabey et al. 1969), sigmoid (Hagin et al. 1976, Lees and Quastel 1946), logistic (Quastel and Scholefield 1951, Stojanovic and Alexander 1958) and Michaelis-Menten (Ardakani et al. 1973, 1974, Laudelout et al. 1977, McLaren 1970, Nishio and Furusaka 1971).

Of these, Michaelis-Menten kinetics provides the most versatile framework since it can be either zero or first order depending on substrate concentration. It is also easiest to justify from a theoretical standpoint since 1) nitrification in soils is universally attributed to wasted metabolism by *Nitrosomonas* and *Nitrobacter*, 2) oxidation of ammonium and nitrate by these microorganisms in solution culture has been found to conform to classical microbial kinetics (Boon and Laudelout 1962, De Leval and Ramacle 1976, Knowles et al. 1965, Laudelout and van Tichelen 1960, Shah 1975, Stratton 1966, Laudelout et al. 1974), and 3) the most widely accepted model for microbial growth is also based on Michaelis-Menten kinetics (Monod 1949).

Since the pioneering series of papers by McLaren (1969, 1970) nearly 10 years ago, there appears to have been little progress in the description of nitrification in soils. Other investigators have considered temperature, pH, aeration, and moisture effects and dealt with them on an empirical basis (Beek and Friessel 1973, Hagin et al. 1976, Sabey et al. 1969). The objective of this report is to review previous work and to present a basis for systematic treatment of the effects of pH and temperature on nitrifier growth and activity in soils.

To review briefly the microbial kinetics used throughout this report, the utilization of a substrate by a bacterial culture is represented as

$$k = \frac{k_{max} \cdot S}{K_m + S} \qquad (1)$$

where k = the observed rate of disappearance of the substrate
k_{max} = a maximum rate attained when the substrate is not limiting
S = the substrate concentration
K_m = a Michaelis constant*

Similarly, growth rates of bacterial populations are represented as

$$\mu = \frac{\mu_{max} \cdot S}{K_m + S} \qquad (2)$$

where μ and μ_{max} are observed and maximum growth rates, respectively, and K_m and S have the same significance as noted above.

Equation 1 assumes a constant bacterial population. To make it applicable to situations in which the population is changing, as in nitrifier growth, the term k_{max} is defined for a single bacterium and multipled

*For a discussion of the concept of Michaelis constant the reader is referred to standard texts of biochemistry.

by the number of bacteria N in the culture at any time:

$$k = \frac{k_{max} \cdot N \cdot S}{K_m + S}. \quad (3)$$

These are calculated from an initial number N_0 and the time t according to Monod (1949):

$$N = N_0(2)^{\mu t}. \quad (4)$$

The growth term μ can also be negative when bacteria are dying due to lack of substrate, for example. However the basic assumptions do not include cell death. This will be discussed in a subsequent report.

LITERATURE REVIEW

Effect of temperature on nitrification

A temperature effect can be ascribed to three of the parameters in the equations for growth and substrate utilization k_{max}, μ_{max}, and K_m. This is because all involve chemical reaction rates (according to enzyme kinetic theory K_m is the equilibrium constant for the dissociation of the substrate-active site complex and therefore the ratio of two rate constants). Experimentally, these parameters appear to obey the expected Arrhenius temperature dependence in the range of nitrifier viability (Boon and Laudelout 1962, Laudelout and van Tichelen 1960, Wong-Chong and Loehr 1975). Logarithmic dependence on T over this range has also been observed (Knowles et al. 1965).

Growth rate constants

Growth rates for ammonium and nitrite oxidizers in pure culture have been reported by a number of investigators. Several investigators have also reported growth rates in solution mixed culture and in soil. We have compiled the available data in Tables 1 and 2. In some cases data have been estimated from figures in the original articles. All growth rates are reported as generation or doubling rates (eq 4). The data Knowles et al. (1965) obtained for river water cultures are plotted in Figure 1 showing the logarithmic dependence on temperature. The data of Buswell et al. (1954) for cultures from trickling filter effluents are shown for comparison. Strictly speaking, the latter are not growth rates constants (μ_{max}) but observed growth rates (μ). However, since they were obtained under non-substrate limiting conditions (NH_4^+-N >> K_m), for practical purposes they are the same. Agreement between these two sets of data is quite good. Also these agree with the results of Skinner and Walker (1961), Engel and Alexander (1958), and Loveless and Painter (1968) for pure cultures of *Nitrosomonas*. Therefore, we have chosen the best fit to the data reported by Knowles et al. (1965) as representing the growth rate constant of ammonium oxidizers. The equation representing these data in units of days^{-1} is

$$\log \mu_{1\ max} = 0.04231\ T - 0.79436. \quad (5)$$

The growth rates reported for soils (Table 1) are consistently much lower than those for solution cultures, although the substrate concentrations used were apparently adequate to have produced maximum rates. These differences between soils and solution cultures are thought to be due to 1) deficiencies of other nutrients in soil, particularly oxygen, or possibly a slower rate of ammonium diffusion and transport, and 2) inhibition by hydrogen ions. Oxygen limitation is suspect because Ardakani et al. (1975) presented theoretical calculations which showed that the oxygen flux in their perfusion experiments was entirely accounted for by NH_4^+ oxidation during steady-state nitrification of urea in a soil column study. Other workers have shown the importance of soil aggregate size on the rate of nitrification (Nishio and Furusaka 1970 and 1971, Seifert 1962 and 1964). It is conceivable that restricted oxygen diffusion through soil aggregates or microbial films (Pirt 1973, Saunders and Bazin 1973, Wuhrmann 1963) may have led to a slower rate of growth in soils. Nishio and Furusaka (1971) postulated the existence of "active" and "inactive" members of the nitrifier community.

The effect of pH on ammonium oxidation and nitrifier growth will be discussed in detail later. Briefly, the work of Morill and Dawson (1962) shows (Table 1) the extreme sensitivity of growth to soil pH, the generation time tripling in a change of pH from 7.6 to 6.2. We suspect that both growth and oxidation are inhibited by the relatively high concentration of H^+ near the surface of soil particles, which may differ considerably from values based on conventional soil pH measurements (McLaren and Packer 1970, Laudelout et al. 1977). A possible mechanism for this inhibition will be suggested.

The data that Knowles et al. (1965) obtained for growth rates of nitrite oxidizers in river water are shown in Figure 2. A logarithmic temperature dependence was again obtained. There are few data for comparison, except in soils, where the growth rates are again lower than expected in the absence of nutrient deficiences and inhibitors (Table 1). The same arguments apply with respect to oxygen deficiency and

Table 1. Growth rates of *Nitrosomonas* sp.

Investigator	*Culture type*	NH_4^+ *(mg/l)*	*pH*	*T (°C)*	*μ (generations/ day)**	*G(h)†*
Macura and Kunc (1965)	Soil (continuous)	74	7.9	28	0.70	34
Macura and Kunc (1965)	Soil (continuous)	35-284	7.9	28	0.91	26
Quastel and Scholefield (1951)	Soil (perfusion)		7.6	21	0.50	48
Stojanovic and Alexander (1958)	Soil (perfusion)	62.5-500	7.7	30	0.75	32
Ardakani et al. (1974)	Soil (perfusion)	75	7.3	~25	0.56	43
Morill and Dawson (1962)	Soil (perfusion)	70	7.6	30	0.71	34
Morill and Dawson (1962)	Soil (perfusion)	70	7.3	30	0.36	66
Morill and Dawson (1962)	Soil (perfusion)	70	7.1	30	0.41	59
Morill and Dawson (1962)	Soil (perfusion)	70	6.6	30	0.25	96
Morill and Dawson (1962)	Soil (perfusion)	70	6.5	30	0.26	91
Morill and Dawson (1962)	Soil (perfusion)	70	6.2	30	0.23	103
Buswell et al. (1954)	Trickling filter effluent	3	8.0-8.5	15	0.79	30
Buswell et al. (1954)	Trickling filter effluent	3	8.0-8.5	20	1.62	15
Buswell et al. (1954)	Trickling filter effluent	3	8.0-8.5	25	2.24	11
Buswell et al. (1954)	Trickling filter effluent	3	8.0-8.5	30	2.74	9
Buswell et al. (1954)	Trickling filter effluent	3	8.0-8.5	32	2.98	8
Downing et al. (1964)	Activated sludge	32	7.5-8.0	20	0.47	51
Skinner and Walker (1961)	Batch culture (Clear)		7.0-7.4	28-32	3.17	8
Skinner and Walker (1961)	Continuous culture (Clear)		7.0-7.4	28-32	2.16	11
Engel and Alexander (1958)	Clear medium		8.0	25	2.16	11
Loveless and Painter (1968)	Jensen strain		8.0	25	2.00	12
Loveless and Painter (1968)	Activated sludge		7.6	25	1.26	19
Knowles et al. (1965)**	Thames River water	8	7.5-7.6	8.3	0.29	83
Knowles et al. (1965)**	Thames River water	3	7.5-7.7	8.6	0.39	62
Knowles et al. (1965)**	Thames River water	8	7.4-7.6	13.9	0.65	37
Knowles et al. (1965)**	Thames River water	3	7.5	14.5	0.84	29
Knowles et al. (1965)**	Thames River water	3	7.6-7.7	22.2	1.44	17
Knowles et al. (1965)**	Thames River water	8	7.7-7.8	23.2	1.73	14
Knowles et al. (1965)**	Thames River water	3-20	7.7	29.4	2.87	8
Knowles et al. (1965)**	Thames River water	17-18	7.3-8.6	19.0	1.01	24
Knowles et al. (1965)**	Thames River water	19-20		27	2.16	11

*All growth rates calculated as $\log_2$ N according to Monod (1949). Conventionally growth rates are expressed in terms of $\log_e$ N. The conversion factor is $\mu_2 = 1.44\ \mu_e$. The generation time is the reciprocal of μ_2.

**μ_{max}.

†G = generation or doubling time.

Table 2. Growth rates of *Nitrobacter* sp.

Investigator	Culture type	NH_4^+ (mg/l)	pH	T (°C)	μ (generations/day)	G(h)*
Ardakani et al. (1973)	Soil (perfusion)	100 (NO_2^-)	7.0	∿25	0.57	42
Ardakani et al. (1974)	Soil (perfusion)	75 (NH_4^+)	7.3	∿25	1.14	21
Quastel and Scholefield (1951)	Soil (perfusion)	35 (NO_2^-)	7.2	21	1.00	24
Quastel and Scholefield (1951)	Soil (perfusion)	70	7.2	21	1.16	21
Quastel and Scholefield (1951)	Soil (perfusion)	140	7.2	21	1.10	22
Quastel and Scholefield (1951)	Soil (perfusion)	280	7.2	21	0.87	28
Macura and Kunc (1965)	Soil (continuous)	17 (NH_4^+)	7.9	28	0.49	49
Macura and Kunc (1965)	Soil (continuous)	35	7.9	28	0.90	27
Macura and Kunc (1965)	Soil (continuous)	73	7.9	28	0.51	47
Macura and Kunc (1965)	Soil (continuous)	142	7.9	28	0.45	53
Morill and Dawson (1962)	Soil (perfusion)	70 (NO_2^-)	7.6	30	0.92	26
Morill and Dawson (1962)	Soil (perfusion)	70 (NO_2^-)	7.3	30	0.96	23
Morill and Dawson (1962)	Soil (perfusion)	70 (NO_2^-)	7.1	30	1.00	24
Morill and Dawson (1962)	Soil (perfusion)	70 (NO_2^-)	6.6	30	1.20	20
Morill and Dawson (1962)	Soil (perfusion)	70 (NO_2^-)	6.5	30	0.92	26
Morill and Dawson (1962)	Soil (perfusion)	70 (NO_2^-)	6.2	30	0.44	54
Stojanovic and Alexander (1958)	Soil (perfusion)	∿ 10 (NO_2^-)	7.7	30	0.82	29
Stojanovic and Alexander (1958)	Soil (perfusion)	∿ 20	7.7	30	0.72	33
Schmidt (1974)	*N. Winogradskyi*, soil incub.	∿700 (NO_2^-)			0.89	27
Knowles et al. (1965)**	Thames River	8 (NH_4^+)	7.5-7.6	8.3	0.72	33
Knowles et al. (1965)**	Thames River	3	7.5-7.7	8.6	0.86	28
Knowles et al. (1965)**	Thames River	8	7.4-7.6	13.9	1.01	24
Knowles et al. (1965)**	Thames River	3	7.5	14.5	1.01	24
Knowles et al. (1965)**	Thames River	3	7.6-7.7	22.2	1.73	14
Knowles et al. (1965)**	Thames River	8	7.7-7.8	23.2	1.87	13
Knowles et al. (1965)**	Thames River	3-20	7.7	29.4	2.85	8
Knowles et al. (1965)**	Thames River	17-18	7.3-8.6	19.0	1.48	16
De Leval and Remacle (1976)	Strain "7" (Laudelout)		7.5	23.0	1.44	17

*G = generation or doubling time.

**μ_{max}.

proton inhibition, in this case believed to be due to the toxic effect of nitrous acid (Boon and Laudelout 1962), as will be discussed in detail later. We have chosen the equation of best fit to the data of Knowles et al. (1965) to represent growth of the nitrite oxidizers. In units of $days^{-1}$

$$\log \mu_{2\ max} = 0.2832T - 0.3665. \quad (6)$$

Oxidation rate constants

Single temperature values of k_{max} for ammonium and nitrite oxidation have been estimated from the literature: 1.3×10^{-13} g N/cell per hour at 30°C for *Nitrosomonas* (Hofman and Lees 1953, Anderson 1965) and 2.2×10^{-13} g N/cell per hour at 20°C for *Nitrobacter* (Srinath et al. 1976, Wong-Chong and Loehr 1975). This calculation is based on a standard cell biomass of 1×10^{-12}g (Pelczar and Reid 1958) though Painter (1970) reports a somewhat lower biomass for *Nitrosomonas* and *Nitrobacter*. The actual biomass of a single cell need not be known. The rate per unit of biomass is sufficient information to do the calculation.

The oxidation rate constants are assumed to have the same temperature dependence as the respective growth rate constants for *Nitrosomonas* and *Nitrobacter*. Thus ammonium and nitrite oxidation rates are determined as a function of temperature from eq 5 and 6 using the fixed values of k_{max} above. In units of g/h per cell or more accurately g/h per picogram of

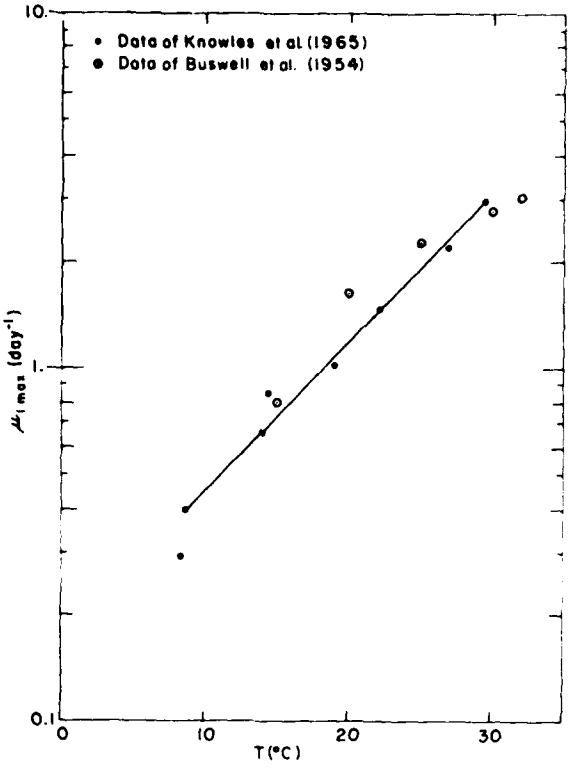

Figure 1. Growth rate constants of Nitrosomonas.

of biomass these are:

$$\log k_{1\ max} = 0.04231T - 14.15536 \qquad (7)$$

$$\log k_{2\ max} = 0.02832T - 13.22398. \qquad (8)$$

These equations give rates of 4.9×10^{-14} and 2.2×10^{-13} g/h per cell, respectively, at a reference temperature of 20°C.

Michaelis constants

The logarithmic temperature dependence of Michaelis constants for nitrite oxidizers given by Knowles et al. (1965) is consistent with data for pure cultures of *Nitrobacter* (Fig. 3, Table 3). Since the agreement among these several investigators is excellent and differs little from the data of Knowles et al. (1965) for river water, we chose the best fit to these data to represent the Michaelis constant for nitrite oxidation:

$$\log K_m = 0.03904T - 0.39217. \qquad (9)$$

The value found by Ardakani et al. (1973) to best fit their data for soil is also of this magnitude after correction for dispersion (Table 3).

Michaelis constants for *Nitrosomonas* are not as well agreed on. There appear to be at least two distinct types of ammonium oxidizers on this basis. The pure cultures of *Nitrosomonas europea* investigated by Meyerhof (1917), Anderson (1965) and Hofman and Lees (1953) have distinctly higher Michaelis constants than those found in sewage treatment plants, river water and probably soil (Table 4). According to the model presented later in this report the observed Michaelis constants for ammonium oxidation are pH dependent; this is the basis for the "pH corrected" values shown in Table 4. The choice of the lower K_m values for soil nitrifiers is consistent with the pH corrected values of Starr et al. (1974) and Ardankani et al. (1974) and may help explain why nitrification occurs at pH 4 in soils but not at this pH in pure culture. In wastewater amended soils one would expect the nitrifiers most able to compete for ammonium (lowest K_m and highest μ_{max}) to succeed those less able. The equation of best fit to Knowles et al. (1965) data

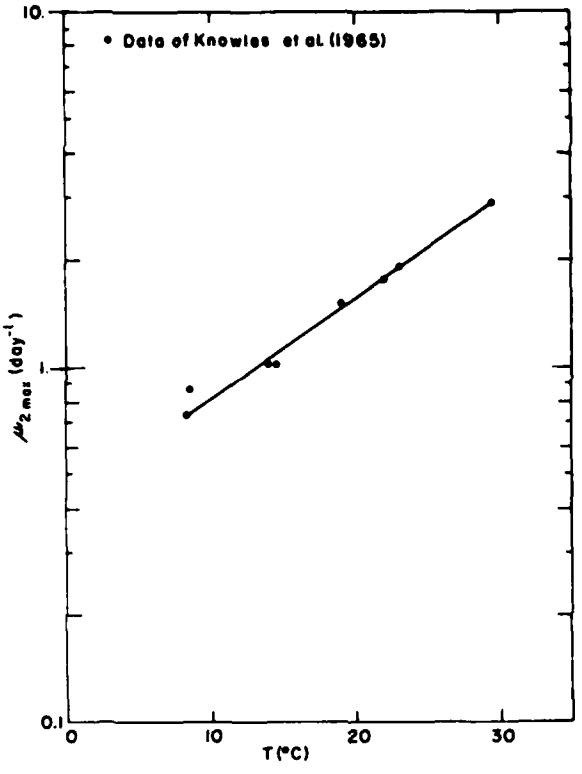

Figure 2. Growth rate constants of Nitrobacter.

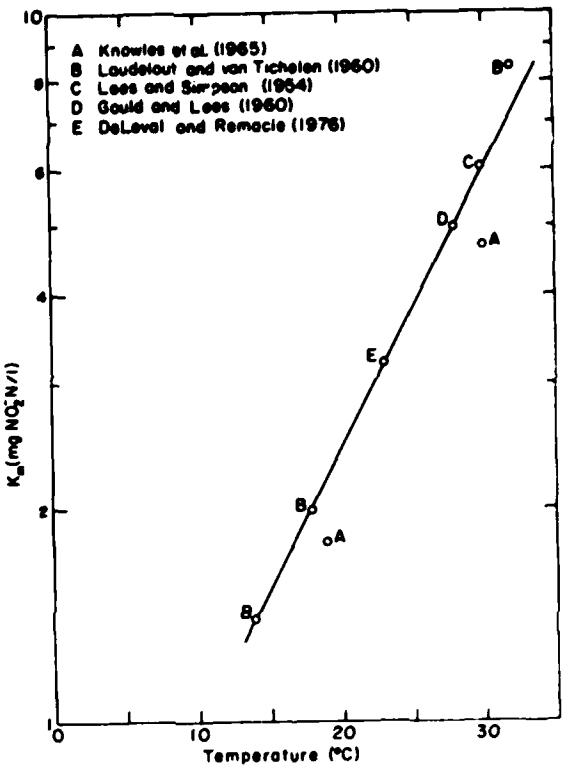

Figure 3. Michaelis constants (NO_2^--N) for Nitrobacter.

corrected to a median pH of 7.7 in their experiments (Fig. 4) is

$$\log K_m = 0.05324T - 1.95351. \quad (10)$$

EFFECT OF DISSOLVED OXYGEN ON NITRIFICATION

Oxygen is not usually considered or is assumed to be non-limiting in discussions of nitrification. Shah (1975), however, has proposed a double substrate form of the Michaelis-Menten equation for treating oxygen and nitrogen simultaneously as limiting nutrients:

$$k = k_{max} \cdot \frac{S_1}{K_{m1}+S_1} \cdot \frac{S_2}{K_{m2}+S_2}. \quad (11)$$

In principle this form of the Michaelis-Menten equation can be expanded to include other limiting nutrients if the Michaelis constants become known. Michaelis constants for oxygen have been determined by a number of investigators (Table 5). The values range from about 0.1 to 1.0 mg O_2/liter and are comparable for liquid and soil cultures.

Boon and Laudelout (1962) have shown that the Michaelis constant for *Nitrobacter* has a logarithmic dependence on the reciprocal of temperature. In principle we can use these data in nitrification models, provided the concentration of oxygen in soil solution is known. This, however, is problematic because it will require a quantitative description of soil aeration (Greenwood 1962 and 1963). Also, the rates of oxygen utilization by other soil biota during nitrification will have to be determined. This is no easy task. We will defer further discussion of soil aeration until our discussion of limiting nitrification rates.

EFFECT OF pH ON NITRIFICATION

Although the occurrence of pH optima for oxidation of ammonium and nitrate has been generally observed, the reported pH optima vary among different investigators. This variation may in part be due to the use of

Table 3. Michaelis constants for nitrite oxidation.

Investigator	*Source*	*T(°C)*	*pH*	K_m (mg NO_2^--N/l)
Lees and Simpson (1954)	*N. winogradskyi*	30	7.8	6.0
Laudelout and Van Tichelen (1960)	*N. winogradskyi*	32	7.8	8.4
Laudelout and Van Tichelen (1960)	*N. winogradskyi*	18	7.8	2.1
Laudelout and Van Tichelen (1960)	*N. winogradskyi*	14	7.8	1.4
Gould and Lees (1960)	*N. winogradskyi*	28	7.8?	5.0
De Leval and Remacle (1976)	Strain "7" (Laudelout)	23	7.5	3.2
Boon and Laudelout (1962)	*N. winogradskyi* cell-free extract	32	7.65	30.8
Boon and Laudelout (1962)	*Intact cells*	32	7.65	22.4
Aleem and Alexander (1958)	Cell-free enzyme	?	?	∿400**
Knowles et al. (1965)	Thames River water	30	7.3-8.6 (7.7)	4.7
Knowles et al. (1965)	Thames River water	19	7.3-8.6 (7.7)	1.8
Ardakani et al. (1973)	Hanford loam soil	∿25	6.6	23
Ardakani et al. (1973)	Hanford loam soil	∿25	6.6	5*

*Ardakani's (1973) data corrected for dispersion (McLaren 1976).
**According to Painter (1970).

Table 4. Michaelis constants for ammonium oxidation.

Investigator	*Source of culture*	*T(°C)*	*pH*	K_m (mg NH_4^+-N/l)	K_m°*
Meyerhof (1917)	*Nitrosomonas* (Omelianski)	18	8.3	11.9	7.9
Hofman and Lees (1953)	*Nitrosomonas europea*	30	8.5	10.8	8.2
Anderson (1965)	*Nitrosomonas europea* (Nicholas and Jones)	30	8.0	16.0	8.0
Knowles et al. (1965)	Thames river water	30	7.3-8.6 (7.7)	2.4	0.8
Knowles et al. (1965)	Thames river water	20	7.3-8.6 (7.7)	0.7	0.2
Buswell et al. (1954)	Trickling filter	30	8.0-8.5	0.3	0.2
Loveless and Painter (1968)	Activated sludge	20	7.9	1.0	0.4
Downing et al. (1964)	Activated sludge	21	7.8	0.2	0.1
Stratton and McCarty (1967)	Activated sludge	25	–	5.6	–
Ardakani et al. (1974)	Hanford loam soil	–	6.6	8.0	0.3
Starr et al. (1974)	Hanford loam soil	20	(A) 6.2 (30-40 cm)	12.5	0.2
Starr et al. (1974)	Hanford loam soil	20	(B) 6.0 (30-40 cm)	18.0	0.2

*pH-correct Michaelis constant assuming $K_{al} = 10^{-8.0}$.
–Data not given.

different strains of organisms. However, some of these differences are reconciled if we consider pH in an inhibitory fashion. What follows is an attempt to reconcile past differences and to advance a more nearly universal model of pH effects on nitrification.

Nitrite oxidation

Boon and Laudelout (1962) found nitrite oxidation by *Nitrobacter* to follow a classical noncompetitive inhibition by nitrous acid, which appears to satisfactorily explain the so-called inhibitory effect of nitrite on its own oxidation. McLaren and Skujins (1963) compared the pH-dependence of nitrite oxidation by *Nitrobacter "agilis"* in inoculated soil with that in solution culture, and found an approximately 0.5 pH unit upward shift in the pH-dependence of the oxidation rate in soil (Fig. 5). This is in agreement with the presence of higher proton concentrations near soil surfaces

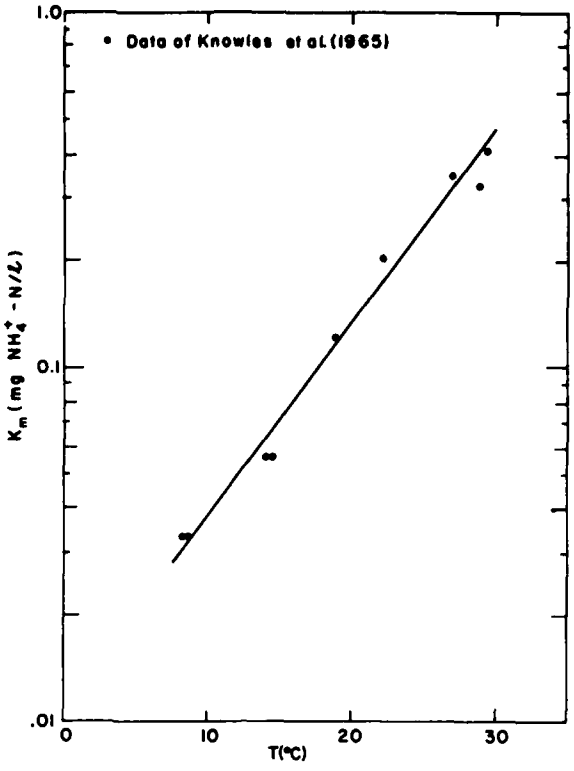

Figure 4. Michaelis constants for ammonium oxidation.

Table 5. Michaelis constants for oxygen.

Investigator	*Source*	*T(°C)*	K_m *(mg* O_2*/l)*
Loveless and Painter (1968)	*Nitrosomonas* culture	20	0.3
Schoberl and Engel (1964)	*Nitrosomonas* culture	30	0.5
Boon and Laudelout (1962)	*Nitrobacter* culture	32	0.5
Schoberl and Engel (1964)	*Nitrobacter* culture	30	1.0
Greenwood (1962)	Soil nitrifiers (mixed culture)	20-23	0.14
Amer and Bartholomew (1951)*	Soil nitrifiers (mixed culture)	30	0.8

*As determined by Shah (1975).

than within bulk soil solution, and McLaren and Packer (1970) have likened enzymes to "molecular pH meters."

Laudelout et al. (1977) recently used a different approach to define pH of acid soils. Hydrogen ion concentrations were calculated from titration data and the soil water content. This method of defining soil pH produced good agreement between rates of chemical decomposition of NO_2^- in soil and in solution as a function of pH. Calculated in this way pH values were 2-3 units lower than the conventionally measured pH of these soils. The spatial variation of pH in soils at the microsite level of microbial action may be even greater than these measurements suggest.

In comparing data on pH effects, it has been convenient and instructive to use semilog plots of rate vs pH (actually log-log if one plots hydrogen ion concentration). This yields linear plots for rates well below the maximum. We suggest that this is not coincidental but is consistent with the mechanism of dissociation of active and inactive enzyme sites (equivalent to

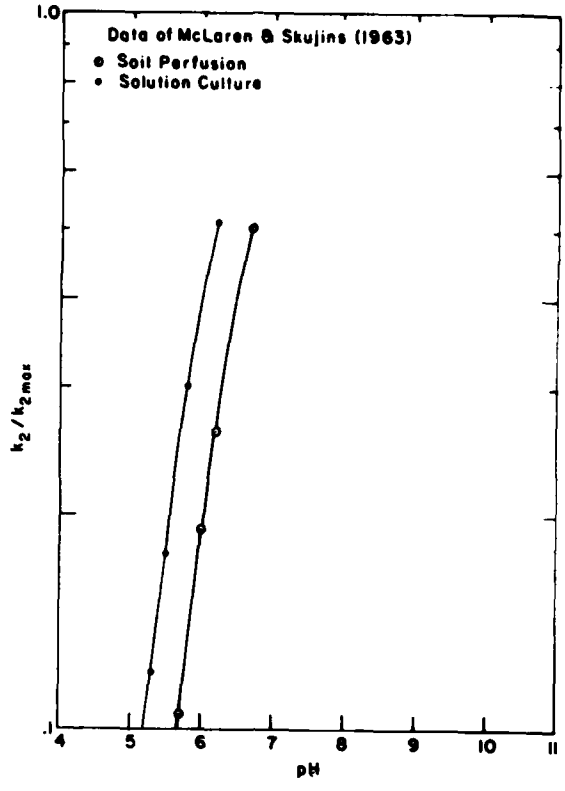

Figure 5. pH dependence of nitrite oxidation by Nitrobacter agilis.

competitive and noncompetitive inhibition) suggested by Boon and Laudelout (1962). A general study of enzyme activities in soils shows similar log-linear relationships to soil reaction (Dutzler-Franz 1977).

Returning to eq 3 and including the effect of nitrous acid inhibition, we find that

$$k_2 = \frac{k_{2max} \cdot N_2 \cdot [NO_2^-]}{(K_{m2} + [NO_2^-]) \left(1 + \frac{[HNO_2]}{K_i}\right)} \qquad (12)$$

where $[HNO_2]$ is the concentration of nitrous acid and K_i the noncompetitive inhibition constant. $[HNO_2]$ is given by

$$[HNO_2] = \frac{[H+]\ [NO_2^-]}{K_a} \qquad (13)$$

where $[NO_2^-]$ is the nitrite concentration and K_a is the dissociation constant of nitrous acid, $10^{-3.4}$. Substitution gives:

$$k_2 = \frac{k_{2max} \cdot N_2 \cdot [NO_2^-]}{(K_{m2} + [NO_2^-]) \left(1 + \frac{[H^+]\ [NO_2^-]}{K_1\ K_i}\right)}. \qquad (14)$$

Choice of appropriate values for k_{max}/k, K_m and K_i enables one to fit experimental data to the model. Boon and Laudelout (1962) obtained a value for K_i of 189 μg HNO_2-N/liter for intact cells (*Nitrobacter winogradskyi*, Engel strain). However, their value of 22 mg NO_2^--N/l for K_m is abnormally high compared to the results reported here for a number of other investigators (Fig. 3). We reanalyzed their data using a value of 7.2 mg/l at 32°C (eq 9). We obtained the best fit with values of k_{max}/k of 1.16 and K_i of 71 μg HNO_2-N/l. The data of Boon and Laudelout are plotted in Figure 6 with the theoretical curve. It is interesting to compare the data of Morrill and Dawson (1962) for growth of *Nitrobacter* during soil perfusion with nitrite. The relevant equation is analogous to eq 14:

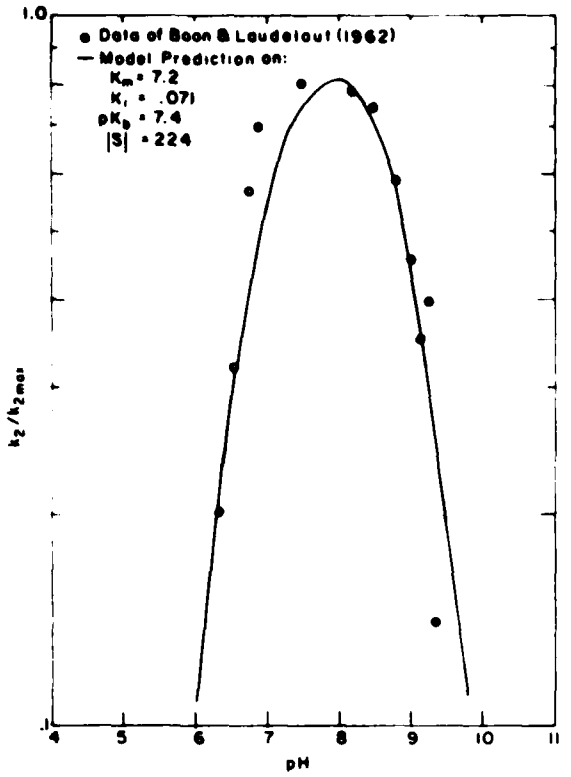

Figure 6. pH dependence of nitrite oxidation (O_2 uptake) by Nitrobacter winogradskyi.

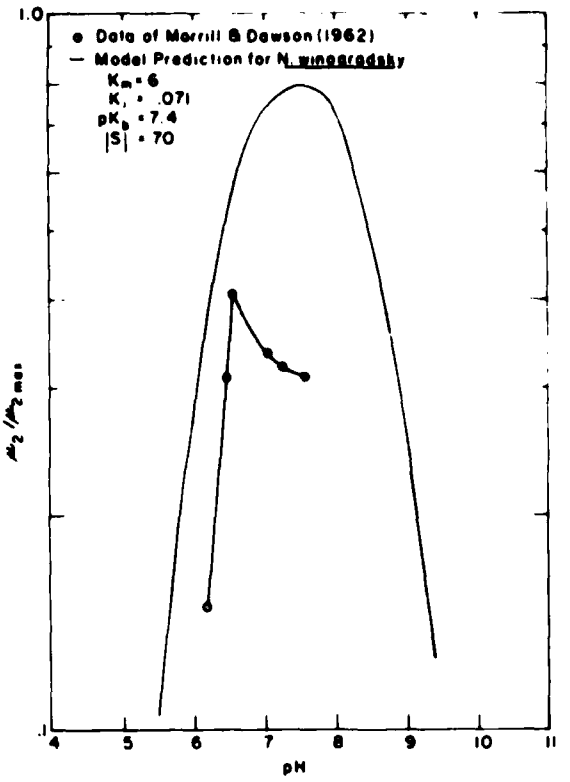

Figure 7. Growth of Nitrobacter *(nitrite oxidation) in soil.*

$$\mu_2 = \frac{\mu_{2max} \cdot N_2 \cdot [NO_2^-]}{(K_{m2} + [NO_2^-]) \left(1 + \left(\frac{[H^+][NO_2^-]}{K_a K_i}\right)\right)} \quad (15)$$

In this case we use a value of 2.97/day for μ_{2max} at 30°C (eq 6). The plotted data (Fig. 7) are shifted to higher pH by about 0.5 unit. This finding is consistent with the results of McLaren and Skujins (1963) (Fig. 5). They noted this effect on *Nitrobacter agilis* in soil. Because the work of Morrill and Dawson represents 116 different soils of widely varying pH in mixed soil culture, the agreement with theory is remarkable. Their results must represent some sort of average condition with respect to hydrogen ion concentration.

We analyzed data from McLaren and Skujins (1963) using eq 14 and found that a K_i of 353 μg HNO_2-N/l gave a satisfactory fit to these data. The theoretical curve then fits satisfactorily with the data for pure *Nitrobacter agilis* culture (Fig. 5), while these data for the *agilis* soil culture lie about 0.5 units higher, as indicated by the authors. If this analysis is correct, then *agilis* is more pH tolerant than *winogradskyi* by 0.5-1.0 pH units*. The available information is thus consistent with assuming that a downward adjustment of soil pH values of 0.5 be made when models for *Nitrobacter* based on solution culture are used.

Boon and Laudelout (1962) explained the pH dependence on the alkaline side of the optimum as being due to competitive inhibition at the active site by hydroxyl ions (OH^-). Other investigators have attributed inhibition at alkaline pH to free ammonia (Aleem and Alexander 1960, Stojanovic and Alexander 1958, Oertli 1972, Anthonisen et al. 1976). It appears that both conclusions are valid since increasing the ammonium level at constant pH caused decreased respiration (Aleem and Alexander 1960), while oxidation is also apparently inhibited by increasing pH in the absence of free ammonia (Boon and Laudelout 1962, Kholdebarin and Oertli 1977).

*Rennie and Schmidt (1977) recently found *agilis* to be numerically dominant in some acid soils.

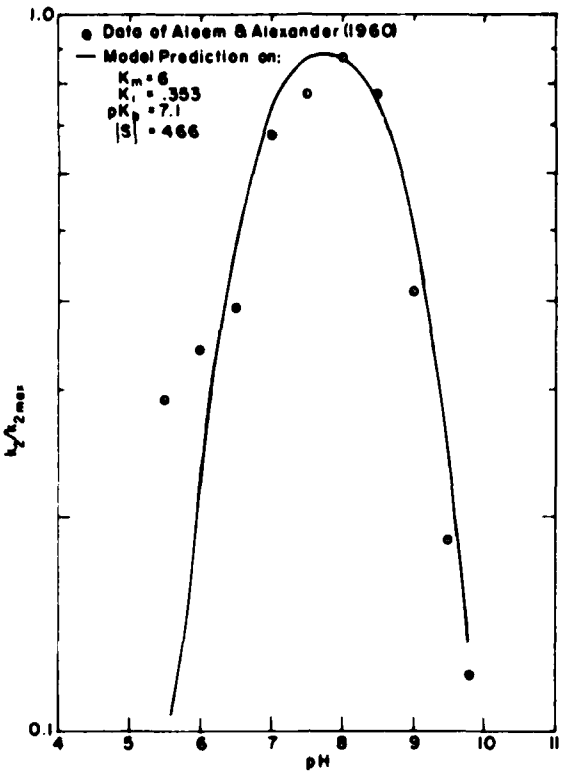

Figure 8. pH dependence of nitrite oxidation (O_2 uptake) by Nitrobacter agilis.

Treating competitive inhibition by OH^-, Boon and Laudelout obtained a pK_b* of 8.3 for their data. The relevant equation for nitrite oxidation in the alkaline region, provided nitrous acid concentration is negligible, is

$$k_2 = \frac{k_{2max} \cdot N_2 \cdot [NO_2^-]}{\left[K_{m2}\left(1+\frac{K_b}{[H^+]}\right)\right]+[NO_2^-]}. \qquad (16)$$

Reanalysis of Boon and Laudelout's (1962) data gave a better fit to their experimental data points with a pK_b of 7.4 (Fig. 6). A comparable set of data for *Nitrobacter agilis* is available (Aleem and Alexander 1960). We obtained a good fit using a pK_b of 7.1 for these data (Fig. 8). This indicates that *agilis* is a little more sensitive to alkaline pH, and less sensitive to acid pH than *winogradskyi* as indicated before.

*K_b is defined as the basic dissociation constant of the active enzyme site.

Although Aleem and Alexander (1960) found that free ammonia was inhibitory to oxygen uptake by *Nitrobacter agilis*, it did not affect the oxidation of nitrite by the cell-free enzyme system. This indicates that the inhibition is noncompetitive and we analyzed their data with a model for noncompetitive enzyme inhibition. The relevant expression when nitrous acid is absent, but when both ammonia and hydroxyl ion are inhibitory, becomes

$$k_2 = \frac{k_{2max} \cdot N_2 \cdot [NO_2^-]}{\left[K_{m2}\left(1+\frac{K_b}{[H^+]}\right)\cdot[NO_2^-]\right]\left(1+\frac{[NH_3]}{K_{i2}}\right)} \qquad (17)$$

where K_{i2} is the inhibition constant for free ammonia. Concentrations of free ammonia in solution can be calculated when the total ammonium added to the system, the pH, and the temperature are known. The NH_3 concentration is calculated from the usual

Table 6. Evaluation of NH_3 inhibition of *Nitrobacter*.

pH	$[NH_3]$	$[NH_4^+]$ (mg N/l)	K_i
9.5	10	4	8
9.5	33.5	13.2	12
9.5	167	66	20
8.4	39	194	80
8.4	79	387	110

equilibrium relations:

$$NH_4^+ \rightleftharpoons NH_3 + H^+ \tag{18}$$

and

$$\frac{[NH_3]\,[H^+]}{[NH_4^+]} = K_a \tag{19}$$

where K_a is the equilibrium constant. Values for K_a at different temperatures can be found in chemistry and physics handbooks; we used the convenient set of values derived by Emerson et al. (1975). We evaluated the linear portions of the oxygen uptake curves of Aleem and Alexander (1960) at pH 8.4 and 9.5 and solved eq 17 for K_{i2}. The results of this analysis were not entirely satisfactory, as values of K_{i2} ranged from 8 to 110 mg NH_3-N/l. The extent of inhibition seemed to depend on the NH_4^+ concentration [with the greater degree of inhibition occurring at the lower NH_4^+ concentrations (Table 6)]. The reason for this is not clear, but may be related to the slight stimulatory effect which ammonium has on cell-free oxidizing systems (Aleem 1959).

Anthonisen et al. (1976) found the zone of NH_3 inhibition of *Nitrobacter* to be in the range of 0.1-1.0 mg NH_3/l. However, they did not take into account the hydroxyl ion inhibition in their experiments. Therefore their apparent values are too low, depending on the concentration of NO_2^- used and the pH. For example, at a concentration of 100 mg NO_2^--N/l, hydroxyl inhibition would result in half-maximal oxidation at a pH of 8.6 with no ammonia present, while at a concentration of 10 mg NO_2^--N/l the half-maximal rate would occur at a pH of 7.2. On the other hand, their zone for nitrous acid inhibition of *Nitrobacter* (0.06-0.75 mg HNO_2-N/l) compares favorably with the values derived here.

Combining known pH effects into one equation gives

$$k_2 = \frac{k_{2max} \cdot N_2 \cdot [NO_2^-]}{\left[K_{m2}\left(1+\frac{K_b}{[H^+]}\right)+[NO_2^-]\right]\left[1+\frac{[HNO_2]}{K_{i1}}+\frac{[NH_3]}{K_{i2}}\right]} \tag{20}$$

The experimental data used to fit pH dependence in *Nitrobacter* are summarized in Table 7.

Ammonium oxidation

The pH dependence of ammonium oxidation has been studied by many investigators with the result that much disagreement exists as to optimum pH and activity at pH values different from the optimum (see Wong-Chong and Loehr 1975). Again, some of the apparent differences can be resolved by considering a model based on classical inhibition phenomena or dissociation of active sites.

Consider the hypothetical equilibrium:

$$EH^+ \overset{K_{a1}}{\rightleftharpoons} E + H^+ \overset{K_{a2}}{\rightleftharpoons} E^- + 2H^+ \tag{21}$$

where E represents the active enzyme site and H^+ the proton in solution. Further, by supposing that only the E form is capable of interacting with ammonium, the following Michaelis-Menten equation can then be derived:

$$k = \frac{k_{max}\,[S]}{K_m\left[1+\frac{[H^+]}{K_{a1}}+\frac{K_{a2}}{[H+]}\right]+[S]}. \tag{22}$$

Thus k would be dependent on the substrate concentration $[S]$ except when $S >> K_m\,(1+[H^+]/K_{a1}+K_{a2}/[H^+])$. We analyzed several experiments from the literature using this model. Values of pK_{a1} and pK_{a2}, derived by trial and error, required initial selection of a value for K_m. According to this model (see also Boon and Laudelout 1962), the observed value K_m^* is related to that theoretically predicted by

$$K_m^* = K_m\left[1+\frac{[H^+]}{K_{a1}}+\frac{K_{a2}}{[H^+]}\right]. \tag{23}$$

An approximate value of K_m^* from Anderson's (1965) data was 16 mg N/l at pH 8.0. Several iterations using different combinations of K_m, K_{a1} and K_{a2} were necessary before finding the optimum combination. Figure 9 shows the result of this exercise where k/k_{max} is plotted vs pH for the optimized combination of

Table 7. Summary of kinetic constants and experimental data used to fit pH dependence in *Nitrobacter*.

Investigator	*Culture type*	*Rate parameter measured*	NO_2^- *conc. (mg/l)**	*T (°C)*	K_m *(mg/l)**	K_i *(mg/l)**	pK_b	*pH at which rate is half-maximal*
Boon and Laudelout (1962)	*Winogradskyi* soln.	O_2 uptake	224	32	7.2	0.078	7.4	6.7, 9.0
Morrill and Dawson (1962)	Mixed soils	Growth as NO_2^- oxidation	70	30	6.0	0.078		6.7
McLaren and Skujins (1963)	*Agilis* soln.	NO_2^- oxidation	198	30	6.0	0.353		6.2
McLaren and Skujins (1963)	*Agilis* soil	NO_2^- oxidation	198	30	6.0	0.353		6.6
Aleem and Alexander (1960)	*Agilis* soln.	O_2 uptake	466	30	6.0	0.353	7.1	6.5, 8.9

*as N

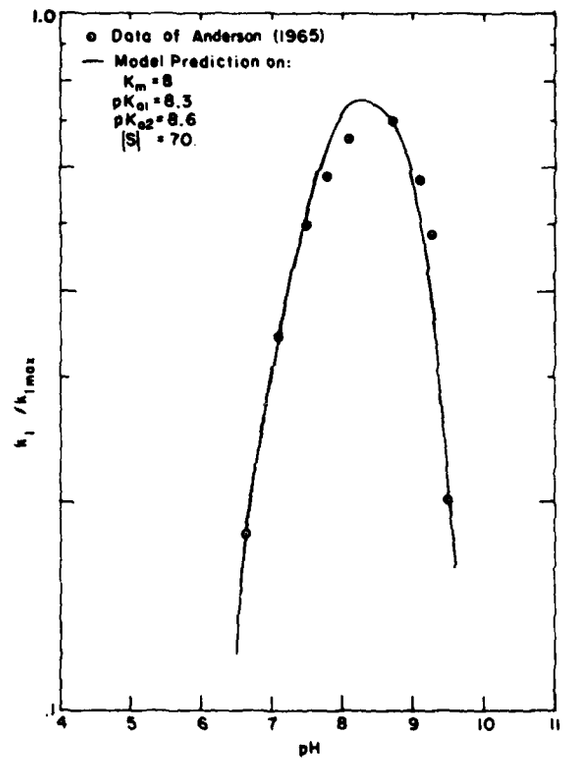

Figure 9. pH dependence of ammonium oxidation by Nitrosomonas europea.

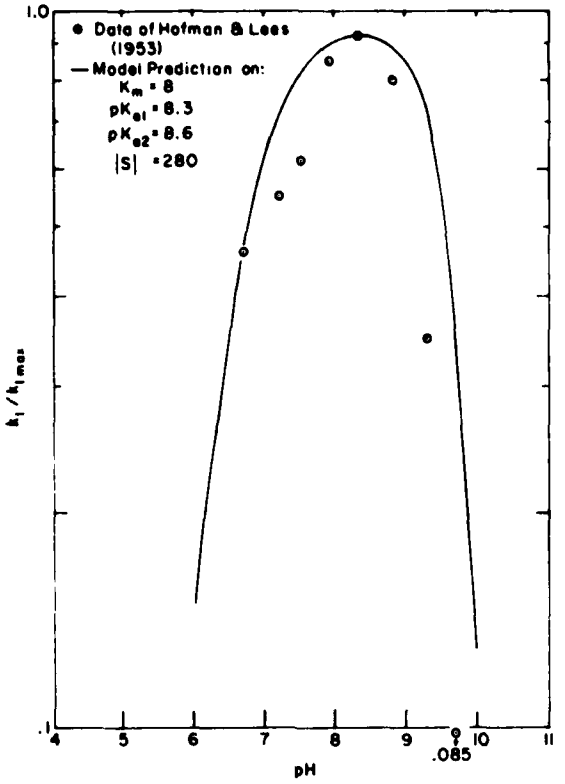

Figure 10. pH dependence of ammonium oxidation (O_2 uptake) by Nitrosomonas europea.

constants. The same set of constants was then used to analyze Hofman and Lees' (1953) data, which are shown in Figure 10. The results are encouraging even though there may have been some additional inhibition by free NH_3 in Hofman and Lees' experiment. As with *Nitrobacter*, however, inhibition of Nitrosomonas by free NH_3 did not follow a simple noncompetitive inhibition model, the values of K_i increasing with pH (data not shown).

The data of Loveless and Painter (1968) are also well fitted by the model using a smaller K_m as discussed earlier (Fig. 11). Unfortunately, several other data sets could not be tested simply because the authors did not report values for substrate concentration. The results are summarized in Table 8.

Application of Michaelis-Menten kinetics to the oxidation of ammonium in soil is complicated by three factors: 1) since NH_4^+ is a cation, its concentration in soil solution depends on a soil-specific equilibrium isotherm and on adsorption kinetics. This makes it impossible to estimate its concentration in soil solution accurately from published experiments, 2) the conventional definition of soil pH has dubious relevance to soil microbial activity (see discussions by Laudelout et al. 1977, McLaren and Packer 1970, McLaren and Skujins 1963, McLaren and Estermann 1957, Harter and Alrichs 1967), and 3) since we are not able to determine if oxygen is limiting, kinetic analysis based on nitrogen is questionable since the Michaelis model presented here assumes a single limiting substrate.

With these factors in mind we have attempted to analyze the experiment reported by Morrill and Dawson (1962) in terms of our model (Fig. 12). The experimental data are plotted using a value for μ_{max} at 30° of 2.83/day from eq 5.

Simulation curves are presented (Fig. 12) for a pK_{a1} of 8.3 (Table 8) and two values of K_m, one typical of river water or sewage cultures and the other of pure *Nitrosomonas* cultures (Table 4). We used an ammonium concentration of 70 mg/l since this is the reported influent concentration. However, whether this concentration was maintained in soil solution is questionable.

One explanation for the shape of the experimental curve is that $[NH_4^+]$ increased with decreasing pH.

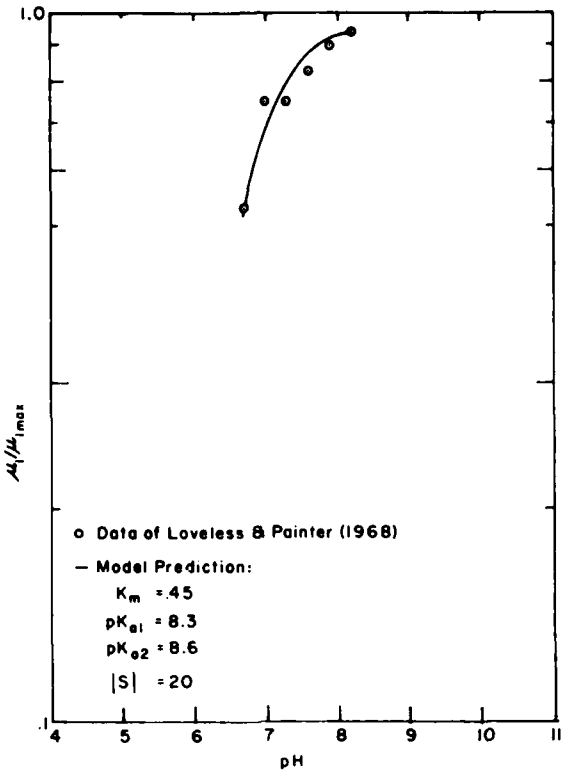

Figure 11. Growth of Nitrosomonas *(ammonium oxidation) in solution.*

Table 8. Summary of kinetic constants and experimental data used to fit pH dependence in *Nitrosomonas*.

Investigator	*Culture type*	*Rate parameter measured*	*NH_4^+ conc. (mg/l)**	*T (°C)*	*K_m (mg/l)**	*pK_{a1}*	*pK_{a2}*	*pH at which rate is half-maximal*
Anderson (1965)	*europea*, soln.	NH_4^+ oxidation	70	30	8.0	8.3	8.6	7.4, 9.2
Hofman and Lees (1953)	*europea*, soln.	O_2 uptake	280	30	8.0	8.3	8.6	6.8, 9.2
Loveless and Painter (1968)	mixed, activated sludge	Growth as NH_4^+ oxidation	20	25	0.45	8.3	8.6	6.7
Morrill and Dawson (1962)	mixed, soils	Growth as NH_4^+ oxidation	70†	30	0.80	8.3	8.6	>7.6

*As N

†Concentration in original soil perfusate.

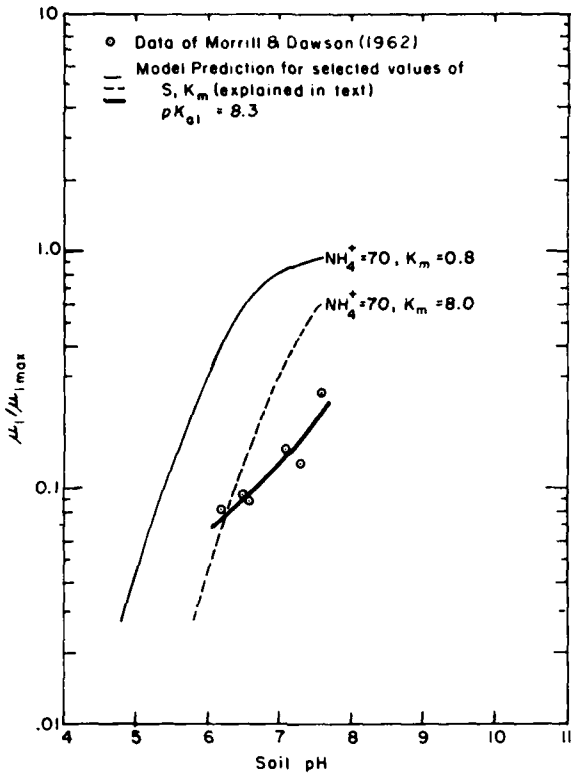

Figure 12. Growth of Nitrosomonas *(ammonium oxidation) in soil.*

A slower rate of nitrification at the lower pH would tend to maintain a higher ammonium concentration in soil solution than at the higher pH, where nitrification was more rapid. Also, ammonium would tend to be displaced from the exchange sites by H^+ at the lower pH. Alternatively, it may be that the measured soil pH deviated from the true microsite H^+ concentration more at higher pH values than at lower ones. The experimental and theoretical curves would then tend to converge as pH was lowered, as indicated by infrared techniques (Harter and Alrichs 1967).

This, then, is presumptive evidence for a pH unit difference of 2 at a soil pH of 7.6, similar to the results obtained with clay minerals (McLaren and Estermann 1957, Harter and Alrichs 1967). However, it is impossible without more experimental data to decide which, if any, of these explanations is correct. This points to the need for better ways of characterizing effective H^+ concentration in soils, such as that suggested by Laudelout et al. (1977). That the oxidation of ammonium in soil should require this kind of analysis is in keeping with the notion that nitrification occurs very close to the surface of soil particles when they are present (Lees and Quastel 1946), even though particulate matter is not required for nitrification.

The observation that nitrification occurs in soils at lower pH than in pure cultures may be a reflection of pH heterogeneity in soil at the microsite level, providing a comfortable niche for nitrifiers in even the more acid soils. However, an alternative explanation is afforded by the analysis of the literature data presented here. This explanation is that a lower Michaelis constant than that observed for pure cultures may be characteristic of ammonium oxidizers in natural habitats (e.g. river water, sewage and soils). This leads to greater tolerance to H^+ if the proposed competitive inhibition model is correct.

LIMITING NITRIFICATION RATES

In adapting Michaelis-Menten models where ammonium-nitrogen is the only limiting substrate, we recognize that limits to growth of the nitrifiers and to

Table 9. Maximum nitrification rates in soils amended with ammonium.

Investigator	Soil type	Air filled porosity (%)	T (°C)	Pore velocity (cm/h)	NH_4^+-N conc. (μg/ml)	k (μg/ml h)
Ardakani et al. 1975	Hanford sandy loam and sand	23	∿25	5.2	100	60
Stewart et al. 1975	Harriston loam	?	24	0.10	740	∿13
Stewart et al. 1975	Harriston loam		24	0.17	740	∿18
Kirda et al. 1974	Columbia silt loam	5	∿25	0.22	200	∿4*
Misra et al. 1974	Columbia silt loam	∿30	20	0.16-0.19	100	∿3*
Starr et al. 1974	Lodi sandy loam	∿20	20	0.25	50	1.6-2.3*
Sabey et al. 1959	Taintor	?	25	0 (incubation)	∿2400	20
Tyler et al. 1959	Salinas clay	?	24	0 (incubation)	∿1600	7
Greenwood 1962	Clay loam	?	30	0 (incubation)	280	3

* Calculated from first order rate constants and initial NH_4^+ conc.

nitrification rates are not implicit. Experimental evidence suggests that these rates are in fact limited by factors other than nitrogen supply. One way of restricting growth (and nitrification rate) is to place an arbitrary limit on the number of nitrifiers. It could be a function of surface area as suggested by McLaren (1969); however there appears to be persuasive evidence that the limitation is due more to rate of oxygen supply than to a surface area requirement for growth *per se*. The view that there is a surface area limitation was challenged by Saunders and Bazin (1973). These investigators preferred the nutrient diffusion-active layer theory of Pirt (1973), in which diffusion of essential nutrients is said to control the size of the active biomass layer. For aerobic processes, such as nitrification, oxygen was assumed to be the limiting nutrient (Pirt 1973). The presence of an active and inactive biomass during nitrification in soils is supported by the experiments of Nishio and Furusaka (1970 and 1971), who advanced a theory similar to Pirt's to account for nitrification in soil aggregates. Seifert (1964) found the nitrification rate to vary inversely as the log of the diameter of the aggregates. Greenwood (1962) advanced an equation based on Fick's law of diffusion which approximately accounted for the relative proportion of aerobic and anerobic zones in soil crumbs under different oxygen partial pressures.

Aeration has been demonstrated to be limiting in solution culture (Skinner and Walker 1961). It is more difficult to ascertain whether oxygen was limiting in reported soil incubation and perfusion experiments. Theoretical calculations of the flux of oxygen into soils during steady-state perfusion with 100-ppm urea solution have shown that the entire flux is consumed in nitrification (Ardakani et al. 1975). Also, even though the oxygen concentration in bulk soil solution may exceed the Michaelis constant for the reaction, the 0_2 concentration at the microsites of nitrification is problematical. Theoretical and experimental evidence shows that the rate of nitrification is affected markedly by the size of soil aggregates (Greenwood 1962 and 1963, Seifert 1962 and 1964, Nishio and Furusaka 1970 and 1971), as was predicted on the basis of diffusion theory in soils. The effects of moisture on nitrification are also in part due to their influence on the rate of oxygen diffusion (Seifert 1962, also see Hattori 1973, p. 307).

In reviewing the literature we find the maximum nitrification rates observed in soil perfusion studies appear to be nearly as high as those in solution culture. Ardakani et al. (1975) observed a maximum *k* of 60 μg/h per ml soil solution during perfusion with 100 ppm-urea. These were somewhat idealized conditions, however, as the column contained 90% sand mixed with 10% Hanford sandy loam, the air-filled porosity (23%) and the solution pore velocity were also high (52 mm/h), and only aggregates smaller than 2 mm were used. Nevertheless, this rate compares favorably with the limiting rate for ammonium oxidation in batch pure culture of 110 μg/h per ml (Wong-Chong and Loehr 1975). Somewhat lower values for ammonium oxidation can be calculated from the data of other investigators. These are summarized in Table 9.

The soil perfusion data for nitrite oxidation of Nishio and Furusaka (1971) show a maximum rate of 45.5 μg N/h g soil at 25° or about 170 μg/h ml soil solution for intact soil. However, dispersion of the soil aggregates by shaking resulted in a limiting rate of 100 μg/h g

or about 370 μg/h ml, comparing well with one of 300 μg/h ml batch culture at 20°C calculated from data given by Wong-Chong and Loehr (1975). These comparisons lend credence to the extrapolation of solution culture data to make predictions of nitrification rates in soil.

This concludes the analysis of the literature and preliminary considerations to building of the mathematical model. In a subsequent report we will describe a computerized model based on the information developed here. This will include provisions for maximum oxygen utilization by the nitrifiers and for cell maintenance and death under nitrogen starvation.

LITERATURE CITED

Aleem, M.I.H., and M. Alexander (1960) Nitrition and physiology of *Nitrobacter agilis*. *Applied Microbiology*, vol. 8, p. 80-84.

Aleem, M.I.H., and M. Alexander (1958) Cell-free nitrification by *Nitrobacter*. *Journal of Bacteriology*, vol. 76, p. 510-514.

Aleem, H. (1959) The physiology and chemoautotrophic metabolism of *Nitrobacter agilis*. Ph. D. thesis, Cornell University (unpublished).

Amer, F.M., and W.V. Bartholomew (1951) Influence of oxygen concentration in soil air on nitrification. *Soil Science*, vol. 72, p. 215-219.

Anderson, J.H. (1965) Studies on the oxidation of ammonia by *Nitrosomonas*. *Biochemical Journal*, vol. 95, p. 688-698.

Anthonisen, A.C., R.C. Loehr, T.B.S. Prakasam, and E.G. Srinath (1976) Inhibition of nitrification by ammonia and nitrous acid. *Journal of the Water Pollution Control Federation*, vol. 48, no. 5, p. 835-852.

Ardakani, M.S., M.G. Volz and A.D. McLaren (1975) Consecutive steady state reactions of urea, ammonium and nitrite nitrogen in soil. *Canadian Journal of Soil Science*, vol. 55, p. 83-91.

Ardakani, M.S., J.T. Rehbock and A.D. McLaren (1974) Oxidation of ammonium of nitrate in a soil column. *Soil Science Society of America, Proceedings*, vol. 38, p. 96-99.

Ardakani, M.S., J.T. Rehbock and A.D. McLaren (1973) Oxidation of nitrite to nitrate in a soil column. *Soil Science Society of America, Proceedings*, vol. 37, p. 53-56.

Beek, J. and M.J. Frissel (1973) Simulation of nitrogen behavior in soils. Centre for Agricultural Publishing and Documentation, Vageningen, Holland.

Boon, B. and H. Laudelout (1962) Kinetics of nitrite oxidation by *Nitrobacter* winogradskyi. *Biochemical Journal*, vol. 85, p. 440-447.

Buswell, A.M., T. Shiota, N. Lawrence and I. Van Meter (1954) Laboratory studies on the kinetics of the growth of *Nitrosomonas* with relation to the nitrification phase of the B.O.D. test. *Applied Microbiology*, vol. 2, p. 21-25.

Cameron, D.R. and C.G. Kowalenko (1976) Modelling nitrogen processes in soil: Mathematical development and relationships. *Canadian Journal of Soil Science*, vol. 56, p. 71-78.

De Leval, J. and J. Remacle (1976) Chapter 18: The influence of environmental factors on nitrification. In *Environmental Biogeochemistry*, vol. 1, Ann Arbor, Michigan: Ann Arbor Science Publishers, Inc., p. 259-269.

Downing, A.L., H.A. Painter and G. Knowles (1964) Nitrification in the activated-sludge process. *Journal of the Institute of Sewage Purification*, vol. 130, no. 2, p. 130-158.

Duffy, J., C. Chung, C. Boast and M. Franklin (1975) A simulation model of biophysiochemical transformations of nitrogen in tile-drained corn belt soil. *Journal of Environmental Quality*, vol. 4, no. 4, p. 477-486.

Dutzler-Franz, G. (1977) Der Einfluss einiger chemischer and physikalischer Bodenmerkmale auf die Enzymaktivität verschiedener Bodentypen. *Zeitschritt für Planzenerährung und Bodenkunde*, vol. 140, p. 329-350.

Emerson, K., R.C. Russo, R.E. Lund and R.V. Thurston (1975) Aqueous ammonia equilibrium calculations: Effects of pH and temperature. *Journal of the Fisheries Research Board of Canada*, vol. 32, p. 2379-2383.

Engel, M.S., and M. Alexander (1958) Growth and autotrophic metabolism of nitrosomonas europea. *Journal of Bacteriology*, vol. 76, p. 217-222.

Ghiorse, W.C. and M. Alexander (1977) Effect of nitrogen dioxide on nitrite oxidation and nitrite-oxidizing populations in soil. *Soil Biology and Biochemistry*, vol. 9, p. 353-355.

Gould, G.W., and H. Lees (1960) The isolation and culture of the nitrifying organisms: Part I. Nitrobacter. Canadian Journal of Microbiology, vol. 6, p. 299-307.

Greenwood, D.J. (1962) Nitrification and nitrate dissimilation in soil. II. Effect of oxygen concentration. *Plant and Soil*, vol. 17, p. 378-391.

Greenwood, D.J. (1963) Nitrogen transformations and the distribution of oxygen in soil. *Chemistry and Industry*, p. 799-802.

Hagin, J., A. Amerberger, G. Kruh and E. Segall (1976) Comparison of nitrate leaching results in a lysimeter experiment to those predicted by a simulation model and estimates of influences of varying parameters on simulation results. *Zeitschrift für Planzenerährung und Bodenkunde*, vol. 4, p. 457-464.

Harter, R.D., and J.L. Alrichs (1967) Determination of clay surface acidity by infrared spectroscopy. *Soil Science Society of America, Proceedings*, vol. 32, p. 30-33.

Hattori, T. (1973) *Microbial life in the soil*. New York: Marcel Dekker Inc.

Hofman, T., and H. Lees (1953) The biochemistry of the nitrifying organisms. 4: The respiration and intermediary metabolism of *Nitrosomonas*. *Biochemical Journal*, vol. 54, p. 579-583.

Kholdebarin, B., and J.J. Oertli (1977) Effect of pH and ammonia on the rate of nitrification of surface water. *Journal of Water Pollution Control Federation*, vol. 49, no. 7, p. 1688-1692.

Kirda, C., J.L. Starr, C. Misra, J.W. Biggar, and D.R. Nielsen (1974) Nitrification and denitrification during miscible displacement in unsaturated soil. *Soil Science Society of American, Proceedings*, vol. 38, p. 772-776.

Knowles, G., A.L. Downing, and M.J. Barrett (1965) Determination of kinetic constants for nitrifying bacteria in mixed culture, with the aid of an electronic computer. *Journal of General Microbiology*, vol. 38, p. 263-278.

Laudelout, H., L. Germain, P.F. Chabalier, and C.N. Chiang (1977) Computer simulation of loss of fertilizer nitrogen

through chemical decomposition of nitrite. *Journal of Soil Science*, vol. 28, p. 329-339.

Laudelout, H., R. Lambert, J.L. Fripiat, and M.L. Pham (1974) Effet de la temperature sur la vitesse d'oxydation de l'ammonium en nitrate par des cultures mixtes de nitrifiants. *Annales de Microbiologie*, vol. 125, p. 75-84.

Laudelout, H., and L. van Tichelen (1960) Kinetics of the nitrite oxidation by *Nitrobacter winogradskyi*. *Journal of Bacteriology*, vol. 79, p. 39-42.

Lees, H., and J.H. Quastel (1946) Biochemistry of nitrification in soil. *Biochemical Journal*, vol. 40, p. 803-815.

Lees, H., and J.R., Simpson (1954) The biochemistry of the nitrifying organisms. 5. Nitrite oxidation by *Nitrobacter*. *Biochemical Journal*, vol. 65, p. 297-305.

Loveless, J.E., and H.A. Painter (1968) The influence of metal ion concentrations and pH value on the growth of a *Nitrosomonas* strain isolated from activated sludge. *Journal of General Microbiology*, vol. 52, p. 1-14.

McLaren, A.D. and E.F. Estermann (1957) Influence of pH on the activity of chymotrypsin at a solid-liquid interface. *Archives of Biochemistry & Biophysics*, vol. 68, p. 157-160.

McLaren, A.D., and L. Packer (1970) Some aspects of reactions in heterogeneous systems. *Advances in Enzymology*, vol. 33, p. 245-308.

McLaren, A.D., and J.J. Skujins (1963) Nitrification by *Nitrobacter* agilis on surfaces and in soil with respect to hydrogen ion concentrations. *Canadian Journal of Microbiology*, vol. 9, p. 729-731.

McLaren, A.D. (1976) Rate constants for nitrification and denitrification in soils. *Radiation and Environmental Biophysics*, vol. 13, p. 43-48.

McLaren, A.D. (1970) Temporal and vectoral reactions of nitrogen in soil: A review. *Canadian Journal of Soil Science*, vol. 50, p. 97-109.

McLaren, A.D. (1969) Nitrification in soil: Systems approaching a steady state. *Soil Science Society of America, Proceedings*, vol. 33, p. 551-556.

Macura, J., and F. Kunc (1965) Continuous flow method in soil microbiology. III. Biological immobilization of nitrogen and phosphrous. *Folia Microbiologia*, vol. 10, p. 125-135.

Mehran, M. and K.K. Tanji (1974) Computer modeling of nitrogen transformation in soils. *Journal of Environmental Quality*, vol. 3, p. 391-396.

Meyerhof, O. (1917) Untersuchungen über den Atmungsvorgang nitrifizierender Bacterien. III. Die Atmung des Nitritbildners und ihre Beeinflussung durch chemische Substanzen. *Pflüger's Archiv*, vol. 166, p. 240-280.

Misra, C., D.R. Nielsen and J.W. Biggar (1974) Nitrogen transformation in soil during leaching: II. Steady-state nitrification and nitrate reduction. *Soil Science Society of America, Proceedings*, vol. 38, p. 294-299.

Monod, J. (1949) The growth of bacterial cultures. *Annual Review of Microbiology*, vol. 3, p. 371-394.

Morrill, L.G., and J.E. Dawson (1962) Growth rates of nitrifying chemoautotrophs in soil. *Journal of Bacteriology*, vol. 83, p. 205-206.

Nishio, M., and C. Furusaka (1970) Distribution of nitrifying bacteria in soil aggregates. *Soil Science and Plant Nutrition*, vol. 16, no. 1, p. 24-29.

Nishio, M. and C. Furusaka (1971) Kinetic study of soil percolated with nitrite. *Soil Science and Plant Nutrition*, vol. 17, p. 61-67.

Oertli, J.J. (1972) The effect of pH and supply of reduced inorganic nitrogen on nitrification of lake water. *Memorie de U'Istituto Italiano di Idrobiologia*, vol. 29, p. 229-240.

Painter, H.A. (1970) A review of literature on inorganic nitrogen metabolism in microorganisms. *Water Research*, vol. 4, p. 393-450.

Pelczar, M.J. and R.D. Reid (1958) *Microbiology*. New York: McGraw-Hill Book Company.

Pirt, S.J. (1973) A quantitative theory of the action of microbes attached to a packed column relevant to trickling filter effluent purification and to microbial action in soil. *Journal of Applied Chemistry and Biotechnology*, vol. 23, p. 389-400.

Quastel, J.H., and P.G. Scholefield (1951) Biochemistry of nitrification in soil. *Bacteriology Review*, vol. 15, p. 1-53.

Rennie, R.J. and E.L. Schmidt (1977) Autecological and kinetic analysis of competition between strains of *Nitrobacter* in soils. In *Soil organisms as components of ecosystems* (U. Lohm and T. Persson, eds.), Proceedings, 6th International Colloquium on Soil Zoology, Ecological Bulletin, Stockholm, Sweden, p. 431-441.

Sabey, B.R., L.R. Frederick and W.V. Bartholomew (1959) The formation of nitrate from ammonium nitrogen in soils. III. Influence of temperature and initial population of nitrifying organisms on the maximum rate and delay period. *Soil Science Society of America Proceedings*, vol. 23, p. 462-465.

Sabey, B.R., L.R. Frederick and W.V. Bartholomew (1969) The formation of nitrate from ammonium nitrogen in soils. IV. Use of the delay and maximum rate phases for making quantitative predictions. *Soil Science Society of America, Proceedings*, vol. 33, p. 276-278.

Saunders, P.T., and M.J. Bazin (1973) Attachment of microorganisms in a packed column: Metabolite diffusion through the microbial film as a limiting factor. *Journal of Applied Chemistry and Biotechnology*, vol. 23, p. 847-853.

Schmidt, E.L. (1974) Quantitative autecological study of microorganisms of soil by immunofluorescence. *Soil Science*, vol. 118, p. 141-149.

Schoberl, P. and H. Engel (1964) Das Verhalten der Nitrifizierenden Bacterien gegenuber gelöstern Sauerstoff. *Archiv für Mikrobiologie*, vol. 48, p. 393-400.

Seifert, J. (1962) Influence of soil structure and moisture content on number of bacteria and degree of nitrification. *Folia Microbiologia*, vol. 7, p. 234-238.

Seifert, J. (1964) Influence of the size of soil structural aggregates on the degree of nitrification. *Folia Microbiologia*, vol. 9, p. 115-120.

Shah, D. (1974) Removal of phosphorus and nitrogen from wastewater by spray irrigation of land. Ph.D. Thesis, Michigan State University (unpublished).

Skinner, F.A., and N. Walker (1961) Growth of *Nitrosomonas* europea in batch and continuous culture. *Archiv für Mikrobiologie*, vol. 38, p. 339-349.

Srinath, E.G., R.C. Loehr and T.B.S. Prakasam (1976) Nitrifying organism concentration and activity. *ASCE, Journal of the Environmental Engineering Division*, vol. 102, p. 449-463.

Starr, J.L., F.E. Broadbent and D.R. Nielson (1974) Nitrogen transformation during continuous leaching. *Soil Science Society of America, Proceedings*, vol. 38, p. 283-289.

Stewart, N.E., C.T. Corke, E.G. Beauchamp and L.R. Webber (1975) Nitrification of sewage sludge using miscible displacement and pertusion techniques. *Canadian Journal of Soil Science*, vol. 55, p. 467-472.

Stojanovic, B.J., and M. Alexander (1958) Effect of inorganic nitrogen on nitrification. *Soil Science*, vol. 86, p. 208-215.

Stratton, F.E. (1966) Nitrification effects on oxygen resources in streams. Ph.D. Thesis, Stanford University (unpublished).

Stratton, F.E. and P.L. McCarty (1967) Prediction of nitrification effects on the dissolved oxygen balance of streams. *Environmental Science and Technology*, vol. 5, p. 405-410.

Wong-Chong, G.M., and R.C. Loehr (1975) Kinetics of microbial nitrification. *Water Research*, vol. 9, p. 1099-1106.

Wuhrmann, K. (1963) Effects of oxygen tension on biochemical reactions in sewage purification plants. In (W.W. Ekenfelder and J. McCabe, eds) *Proceedings, 3rd Biological Waste Treatment Conference*, Pergamon Press: New York.

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